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A novel immunochromatographic strip test for rapid detection of Cry1Ac and Cry8Ka5 proteins in genetically modified crops

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The cultivation of genetically modified (GM) crops has grown rapidly worldwide. This has led to regulatory authorities implementing strict procedures to monitor and verify the presence and abundance of GM varieties in agricultural crops. Immunochromatographic strip tests have been employed for the detection of transgenic proteins expressed in GM crops as rapid, reliable and cost-effective screening tools. In this study, we developed a novel and sensitive strip test assay, based on a sandwich format, for the identification of Cry1Ac and Cry8Ka5 transgenic proteins. We generated two monoclonal antibodies (mAb), namely 1B1 and 5H4, that bind with high specificity to Cry1Ac and Cry8Ka5 proteins. For the development of strip tests, colloidal gold particles were coated with 1B1 mAb and used as the detector reagent. The 5H4 mAb was sprayed at the test line of a nitrocellulose membrane to serve as a capture reagent and anti-species specific antibodies were used at the control line. The strip test developed was capable of detecting 0.06 μg of Cry1Ac and Cry8Ka5 proteins. For validation of the strip test, GM and non-GM cotton leaf samples were employed. The results indicated that the strip test was capable of distinguishing between GM and non-GM cotton samples, offering potential for use as a rapid and cost-effective screening tool for insect-resistant GM crops expressing Cry1Ac and Cry8Ka5 proteins to control lepidopterans and coleopteran pests, respectively.

Introduction

Genetic transformation has become an important tool for crop improvement.[1] The application of engineering to food and feed crops is widely acknowledged as a useful tool for addressing global agricultural challenges of population growth and climate change.[2] Such agricultural biotechnology has been widely adopted by growers, with GM crops to date with insect resistance or herbicide tolerance traits requiring lower inputs and allowing flexibility in crop management strategies, whilst maintaining or increasing crop yield and quality.[3,4]

GM insect-resistant crops have been developed using specific genes isolated from the naturally occurring soil bacterium *Bacillus thuringiensis* (Bt).[5] These genes encode specific insecticidal proteins known as Cry proteins.[6] The introduction of genetically engineered crops incorporating these insecticidal proteins has provided growers with an opportunity to reduce insecticide application required for the management of lepidopteran, coleopteran and dipteran insect-pests.[7,8] To date, Bt cry genes have been widely applied in GM maize and cotton development.[9] For example, in Brazil it is now possible to find at least 12 varieties of Bt cotton released for cultivation that express the Cry1Ac protein alone or fused to Cry2Ab2 or Cry1F proteins.[10] Now, these GM cotton varieties mainly target the cotton bollworm *Helicoverpa armigera*. In the case of the major coleopteran cotton boll weevil *Anthonomus grandis*, the mutant toxin Cry8Ka5, which offers increased activity against this pest, has recently been proposed for incorporation into this agribusiness commodity in Brazil.[8,11]

Given the rapid development of GM crops and their increased presence in food and feed, public concern over traceability, food safety and potential ecological contamination is becoming widespread.[12] As such, there is an increasing demand for analytical methods for the detection of introduced genes encoding DNA or their expressed protein(s) in transgenic plants.[1,9] Moreover, precise and accurate detection methods are a prerequisite for reliable control of GM crops in the agricultural market.[13] Various methodologies have been employed to

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detect the presence of GM materials in food.[15–20] Recently, a database of GMO detection methods (GMDD) was developed, which summarizes all GMO detection methods developed to date.[20] Commonly employed DNA-based methods include PCR, real-time PCR and DNA chip technology,[18,19] while protein-based methods include immuno-PCR,[14] mass spectrometry[15,16] and near infrared (NIR) spectroscopy.[17] Although these methods are very accurate and sensitive, they are not cost-effective for large-scale analyses. While less expensive protein-based methods, such as enzyme linked immunosorbent assay (ELISA) and Western blot, also offer accuracy and sensitivity, these methods still have limitations, in that they require trained personnel and are unsuitable for on-site testing.[22]

The immunochromatographic (IC) strip test is a well-established diagnostic tool.[23] This technology offers advantages in terms of speed, simplicity and cost-effectiveness when compared to the abovementioned detection methods.[21] Moreover, IC strip tests also offer convenience for on-site testing under field conditions by untrained personnel.[24] Although several IC strips are now commercially available for the detection of transgenic events, no detection kits are available for GM crops carrying the Cry8ka5 protein. Thus, the goal of this study was to develop an IC strip for the simultaneous detection of the Cry1Ac protein, which is found in Bt crops approved for cultivation, and the Cry8Ka5 mutant protein, which has been employed by our group in the development of transgenic cotton.

Materials and methods

Reagents and materials

Goat anti-mouse immunoglobulin G (IgG) conjugated to horseradish peroxidase (HRP), complete and incomplete Freund's adjuvants, chloroauric acid ($HAuCl_4 \cdot 3H_2O$), sodium citrate, polyethylene glycol 4000 (PEG 4000), hypoxanthine-aminopterin-thymidine (HAT) medium, 4-nitrophenyl phosphate and a mouse monoclonal antibody ISO2-1 kit were purchased from Sigma-Aldrich (St. Louis, MO, USA). RPMI1640 medium with L-glutamine and HEPES, fetal bovine serum (FBS), penicillin, streptomycin and rabbit anti-mouse immunoglobulin IgG were purchased from Thermo Fisher Scientific (Rockford, IL, USA). A HiTrap protein G HP affinity column was purchased from GE Healthcare (Uppsala, Sweden). Gelatin (blotting grade), 4-(4-amino-3,5-dimethylphenyl)-2,6-dimethylaniline (TMB) and a protein assay kit were obtained from Bio Rad Laboratories (Hercules, CA, USA). A high-flow nitrocellulose membrane, a sample pad, a conjugate pad and an absorbent pad were obtained from Millipore (Bedford, MA, USA). High binding flat-bottomed polystyrene microplates were obtained from Costar (Corning, MA, USA), and cell culture flasks and plates were from TPP (St. Louis, MO, USA). Murine myeloma Sp2/0-Ag14 cell lines were kindly provided by Dr Sandra Farias from UFRGS, Rio Grande do Sul, Brazil.

Instruments

A XYZ 3050 Biostrip Dispenser and a CM 4000 Cutter were purchased from Bio-Dot (Irvine, CA, USA). A Microplate Reader Spectra Max M2 was obtained from Molecular Devices Corp. (Sunnyvale, CA, USA).

Preparation of the Bt Cry1Ac and Cry8Ka5 toxins. The Cry1Ac protein was produced by heterologous expression in *E. coli* JM109, transformed with the recombinant plasmid pKK223-3 containing the truncated Cry1Ac gene of *B. thuringiensis* subsp. *kurstaki* (HD73), according to the reported method.[25] The activation of the Cry1Ac toxin was performed with trypsin 1 : 50 (w/w) for 2 h, at 37 °C.

Cry8Ka5 protein production was performed by heterologous expression in *E. coli* BL 21(DE3) containing the mutant gene inserted into plasmid pET101/D TOPO, as described by Oliveira.[8]

All toxin samples were purified, identified by 12% SDS-PAGE, quantified *via* the Bradford method[26] using BSA as a standard, and stored at −80 °C.

Production of monoclonal antibodies (mAbs)

Immunization. The Cry1Ac protein was used as an immunogenic molecule for obtaining mAbs. The Cry1Ac protein (250 μg) was added to 0.25 mL sterilized PBS and emulsified with an equal volume of Freund's complete adjuvant. The solution was applied *via* intraperitoneal injections in ten 8 week-old female Balb/c mice, during a three-week interval. Subsequent immunization (boosters) was applied with incomplete Freund's adjuvant. Later, blood samples were removed from the caudal vein of the mice, at intervals after each booster, and then assayed by indirect ELISA for antibody titration. The mouse with the highest serum titration received a final intraperitoneal injection three days before cell fusion.

Experimental procedures were carried out strictly in accordance with the "Administrative Rules for Laboratory Animals in Brasília (DF) state" (2011), and were approved by The Animal Care and Use Committee of EMBRAPA Genetic Resources and Biotechnology (Brasília, DF, Brazil).

Cellular fusion and screening of hybridomas. Cell fusion procedures were performed according to the reported method,[27] with modification. Briefly, spleen cells from the immunized mice were isolated and fused with Sp2/0-Ag14 murine myeloma cell lines at a ratio of 1 : 10 in the presence of 1 mL PEG 4000 solution (0.8 g mL^{-1}). The fused cells were diluted with fresh HAT medium and distributed into a 96-well culture plate. After ten days, hybridoma supernatants were analyzed by ELISA for the presence of antibodies against the activated Cry1Ac protein. Culture supernatants from the cells with the highest absorption value were transferred to 24-well culture plates and again tested. Only clones that maintained higher absorption in ELISA tests were chosen for further selection. Cell suspensions from each well of the 24-well culture plate were diluted in order to obtain one cell per well, and distributed into a 96-well culture plate. Wells containing a single hybridoma were retained for antibody production and further characterization. These cloned hybridoma cells were introduced *via* intraperitoneal injection into Balb/c mice pretreated with an injection of 0.3 mL pristane. The resulting ascites were purified using a protein A affinity column.

ELISA assays

96-well microplates were coated with 1 μg of Cry1Ac or Cry8Ka5 protein in coating buffer (0.05 M carbonate–bicarbonate, pH 9.6) and incubated at 4 °C overnight. The microplates were washed three times with PBST buffer (PBS containing 0.05% (v/v) Tween 20, pH 7.4). To block remaining sites on the wells, a blocking solution (PBST and 3% (w/v) gelatin) was used for 1 h at 37 °C. After subsequent washing, 100 μL of 1 : 2000 dilution of each mAbs were added and incubated for 1 h at 37 °C. The plate was repeatedly washed three times to remove unbound antibodies and then incubated with 1 : 5000 dilution of goat anti-mouse IgG-HRP prepared in PBST (containing 1% gelatin). The microplates were incubated for 1 h at 37 °C, 100 μL of TMB solution was added to each well, and then the plates were incubated for 10 min at room temperature. The reaction was stopped by the addition of 50 μL 2 M sulfuric acid and absorbance values at 450 nm were then determined.

Mapping the binding region of mAbs to Cry1Ac protein. Synthetic peptides (2.5 μM): PT3b (PPRQGFSHRLSHV), PT4c (LGQGEYRTLSST), PT4d (IIRAPMFSWIHRSAE), PT5d (GTEFAYGTSPNL) and PT5e (FRRELTLTVLDI) were produced by the company GenScript (USA). These peptides were coupled to BSA according to the reported method,[28] and then incubated on a 96-well microplate overnight at 4 °C. The same procedures were followed as described in ELISA assays.

Sandwich ELISA. 96-well microplates were coated with 100 μL of capture antibody (mAb) at 1 : 2000 dilution in coating buffer and incubated at 4 °C overnight. Following three repeated washings with PBST, each well was filled with 200 μL of blocking buffer and incubated at 37 °C for 1 h. Afterwards 1 μg Cry1Ac protein was added and incubated at 37 °C for 1 h. Following washing, 100 μL of the detection antibody (mAb) at 1 : 2000 dilution was added to each well and incubated at 37 °C for 1 h. Washes were conducted as in the previous step, 100 μL goat anti-mouse IgG-HRP (1 : 5000 dilution in PBST containing 1% gelatin) was then added to each well and incubated at 37 °C for 1 h. Plates were washed again, and 100 μL TMB solution was finally added to each well. After color development at room temperature for 10 min, the reaction was stopped with 50 μL of 2 M H_2SO_4 and the absorbance value was determined at 450 nm.

Characterization of mAbs

Isotypes of mAbs were obtained using an isotyping kit according to the manufacturer's instructions. Analysis of the cross-reactivity (CR) of antibodies was performed by ELISA, using the Cry8Ka5 recombinant protein as the antigen. The ELISA assay was employed as described previously.

Preparation of colloidal gold labelling of the anti-Cry mAb

Colloidal gold with a mean particle diameter of 40 nm was produced by the reduction of gold chloride with 1% sodium citrate, as previously described.[29] The probe was prepared as previously reported[30] with minor modifications. Prior to conjugation, the optimal pH and amount of antibody for conjugation were determined. Briefly, 150 μg of purified 1B1 mAb was added drop-wise into 10 mL of a colloidal gold solution (pH 9.0) with gentle stirring. The mixture was agitated for 1 h at room temperature using an overhead shaker. BSA was added to block any remaining free binding sites on the surface of the gold particles, and the solution was again incubated for 30 min at room temperature. Unbound proteins were removed by centrifugation (18 500 × *g* for 30 min, 4 °C), the pellet was washed with deionized water and the mixture was again centrifuged. After removal of the supernatant, the pellet was dissolved in 2 mL of BSA solution at 2% and stored at 4 °C until use.

Preparation of the immunochromatographic (IC) test strip

The test strip was prepared by applying 5H4 mAb (0.5 μg μL^{-1}) and anti-mouse IgG (0.5 μg μL^{-1}) to the nitrocellulose membrane at the test and control lines, respectively. With the test and control lines separated by a distance of 5 mm, the reagents were applied in a dot format using a BioDot XYZ 3050 micro-brush at 1 μL cm^{-1}. The total volume dispensed onto the test and control line was 0.5 μL of solution. Therefore, each strip test contained 0.25 μg of 5H4 mAb and anti-mouse IgG at the test and control lines, respectively. The coated membrane was then dried at 37 °C for 30 min. Subsequently, the membrane was blocked with 2% BSA solution to prevent nonspecific adsorption and stored in a desiccator. For the conjugate pad, the airbrush equipment was calibrated to release 10 μL cm^{-1} of 1B1 mAb-gold conjugate. The initial solution of the conjugate used as a detection reagent was at a concentration of 0.075 μg μL^{-1}. For each strip test, the total volume dispensed was 5 μL, corresponding to 0.375 μg of 1B1 mAb. Afterwards, the conjugate pad was then dried at 37 °C for 30 min. The coated membrane, conjugate pad and absorbent pad were assembled and cut lengthways (5 mm × 70 mm) using a guillotine cutter. The sample pad (absorbent paper) and conjugate pad (glass-fiber membrane) were previously treated with blocking solutions (20 mM phosphate buffer, pH 7.4, containing 2% (w/v) BSA, 2.5% (w/v) sucrose, 0.3% (w/v) polyvinylpyrrolidone and 0.02% (v/v) sodium azide) and dried at 37 °C overnight.

IC strip *in vitro* test with Bt Cry1Ac and Cry8ka5 proteins

Purified Cry1Ac and Cry8Ka5 protein samples (2.5 μg mL^{-1}) were used to determine the sensitivity of the strips. The samples were prepared in 0.05 M carbonate–bicarbonate, pH 9.6, buffer and 200 μL of each sample, equivalent at a final concentration of 0.5 μg, were dispensed into the wells of a microplate. Afterwards, each individual strip was dipped into test samples and left for 10 min.

IC strip assay in cotton samples

Cotton leaf samples, GM (Bollgard®), GM (developed by our team) and non-GM (Coker-312), were used to confirm the accuracy of the developed strip test. The GM and non-GM leaf disc samples were ground to a fine powder (100 mg) and homogenized with 1 mL of extraction buffer (PBS with 0.05% (v/v) Tween-20 and 1% PVP-40 and 0.032 mg mL^{-1} trypsin), according to the reported method.[31] The homogenate was agitated for 30 min and incubated at 37 °C for 1 h. Afterwards, 22 μL of 10 mM phenylmethanesulfonyl fluoride (PMSF) was added as the stop solution. These extracts were directly used for strip test evaluation. 200 μL of each sample was dispensed into microplate wells and the strips were dipped into homogenates and incubated at room temperature for 10 min.

Statistical analyses

Data were analyzed using GraphPad InStat™ (GraphPad software, V2.05). ANOVA analyses were performed using the Bonferroni post test and Tukey's multiple comparison tests with confidence intervals of 95%. Values of $p < 0.01$ and $p < 0.05$ were considered statistically significant.

Results and discussion

Recombinant protein production

SDS-PAGE profiles of Cry1Ac and Cry8Ka5 recombinant proteins after expression and purification are shown in Fig. 1. The toxins presented the expected molecular mass (near 70 kDa). The Image Master 2D platinum (v.7.0 GE Healthcare) software was used to estimate the relative percentage of purity of each protein obtained. For both Cry1Ac and Cry8Ka5 proteins, different batches following expression and purification presented relative purities, which ranged from 75% up to 95%. The SDS-PAGE methodology is widely used by chemical companies for analysis of the purity of commercialized proteins.[11]

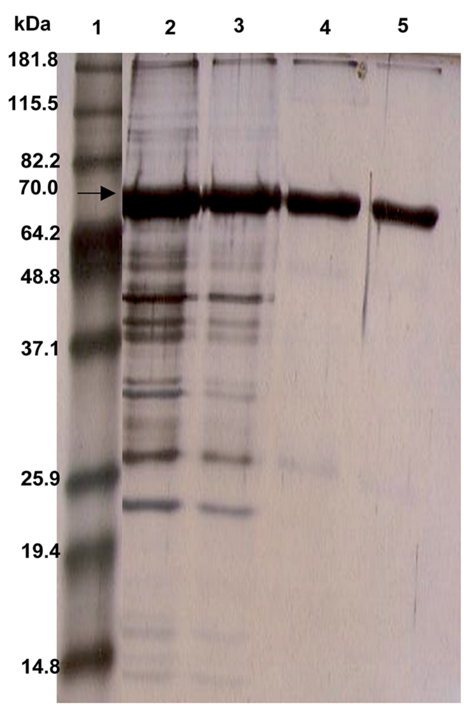

Fig. 1 SDS-PAGE profiles of the heterologous expression products of Cry1Ac and Cry8Ka5 proteins. Lane 1: molecular mass marker (BenchMark™ Pre-Stained Protein Ladder, Invitrogen); lane 2: before purification of the Cry1Ac protein; lane 3: before purification of the Cry8Ka5 protein; lane 4: Cry1Ac toxin after trypsinization and dialysis; lane 5: Cry8Ka5 toxin after purification on the chromatographic affinity (Ni-NTA resin).

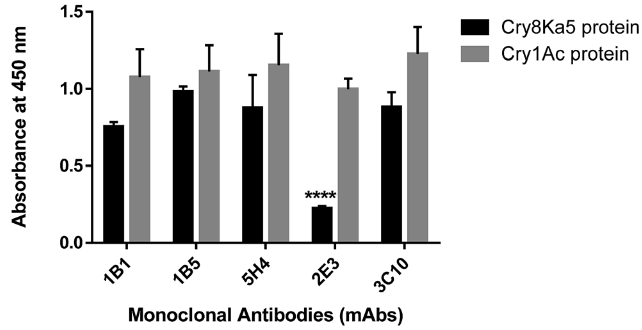

Fig. 2 Evaluation of the cross-interaction of the mAbs anti-Cry1Ac against the Cry8Ka5 recombinant protein by ELISA. High binding EIA/RIA microplates were coated with 1 μg of Cry1Ac or Cry8Ka5 protein and kept overnight at 4 °C. The plates were blocked with 3% gelatin in PBST. An aliquot of 100 μL of each mAbs (1 : 2000) was added for 1 h at 37 °C. The binding was identified using a goat anti-mouse IgG-HRP (1 : 5000) and developed using 100 μL of 4-(4-amino-3,5-dimethylphenyl)-2,6-dimethylaniline (TMB). The reaction was stopped by the addition of 50 μL of 2 M sulfuric acid, and the absorbance was measured at 450 nm. All statistical tests were performed using a two-way ANOVA. ****Denotes $p < 0.01$.

Production of monoclonal antibodies (mAbs)

Antisera from immunized mice showed higher titer values in ELISA assays using the Cry1Ac protein as the antigen than in non-immunized mice. After fusion, cloning and ascites

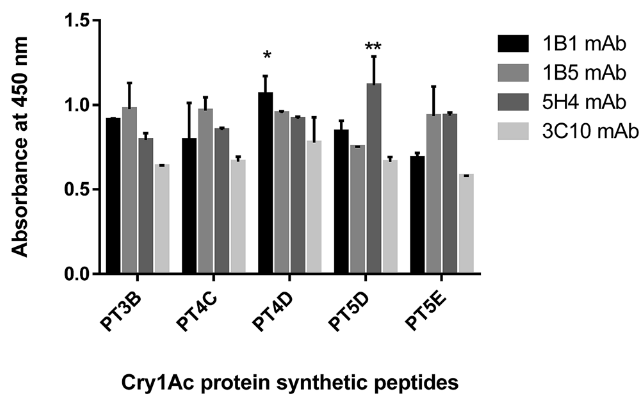

Fig. 3 Epitope mapping of mAbs by ELISA using Cry1Ac toxin synthetic peptides. The peptides coupled with the BSA protein (1 μg) were coated on high binding EIA/RIA microplates and kept overnight at 4 °C. The plates were blocked with 3% gelatin in PBST. An aliquot of 100 μL of each mAb (1 : 2000) was added for 1 h at 37 °C. The binding was identified using a goat anti-mouse IgG-HRP (1 : 5000) and developed using 100 μL of 4-(4-amino-3,5-dimethylphenyl)-2,6-dimethylaniline (TMB). The reaction was stopped by the addition of 50 μL of 2 M sulfuric acid, and the absorbance was measured at 450 nm. All statistical tests were performed using a two-way ANOVA. **Denotes $p < 0.05$.

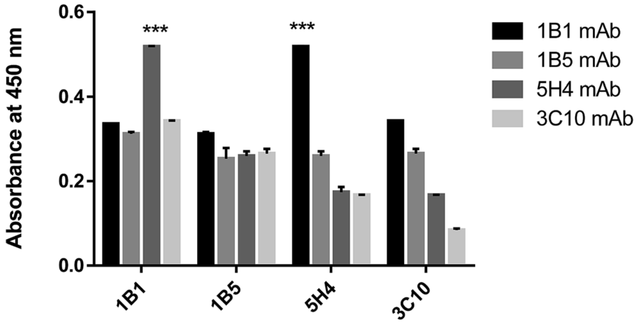

Fig. 4 Evaluation of interaction of mAbs by Sandwich ELISA. High binding EIA/RIA microplates were coated with 100 μL of capture antibody (mAb) at 1 : 2000 dilution in coating buffer at 4 °C overnight. The plates were blocked with 3% gelatin in PBST. Then, 1 μg Cry1Ac protein was added and kept at 37 °C for 1 h. An aliquot of 100 μL of the detection antibody (mAb) at 1 : 2000 dilution was added to each well and incubated at 37 °C for 1 h. Binding was identified using a goat anti-mouse IgG-HRP (1 : 5000) and developed using 100 μL of 4-(4-amino-3,5-dimethylphenyl)-2,6-dimethylaniline (TMB). The reaction was stopped by the addition of 50 μL of 2 M sulfuric acid, and the absorbance was measured at 450 nm. All statistical tests were performed using a two-way ANOVA. ****Denotes $p < 0.01$.

purification, five mAbs were obtained, identified as 1B1, 1B5, 5H4, 2E3 and 3C10. The interaction of each mAb with Cry1Ac or Cry8Ka5 proteins was analyzed by ELISA. The results showed that all mAbs were highly specific to Cry1Ac and were able to recognize Cry8Ka5, with the exception of mAb 2E3 (Fig. 2). Therefore, this mAb was not used in subsequent assessments. These results suggest that the specific mAbs bind to conserved structures among these Cry toxins, as described previously.[34] Previous studies reported a monoclonal antibody produced against a Cry1Ab lepidopteran-specific toxin that also showed cross-reaction with a Cry9 coleopteran-specific toxin.[35] Several mAbs produced against peptide sequences from Cry toxins have been used to elicit the binding site of these proteins with their specific receptors.[32,33,36] It is important to increase knowledge about the mode of action of Cry toxins for target insects. In this study, specific synthetic peptides of the binding region of the cadherin-like receptor from *Helicoverpa armigera* (HaCad) to the Cry1Ac protein (data not shown) were used for epitope mapping of mAbs produced. For this assay, the Cry1Ac protein was not previously activated because the PT5e peptide sequence is localized in the N-terminal domain found on the truncated Cry1Ac protein. As shown in Fig. 3, of the five peptides tested, only the PT4d and PT5d sequences to mAbs 1B1 and 5H4 showed a statically significant difference ($p < 0.05$). These findings suggest that the 1B1 and 5H4 mAbs recognized different binding sites on the Cry1Ac protein. To confirm these results, a sandwich ELISA assay was performed. Pairwise mAb combinations are shown in Fig. 4. When the combination of 5H4 mAb capture antibody and 1B1 mAb as the second antibody was used, an increase in pairing occurred ($p < 0.05$) greater than those observed in other mAb combinations. Hence, these two mAbs were selected and used for the preparation of the strip test.

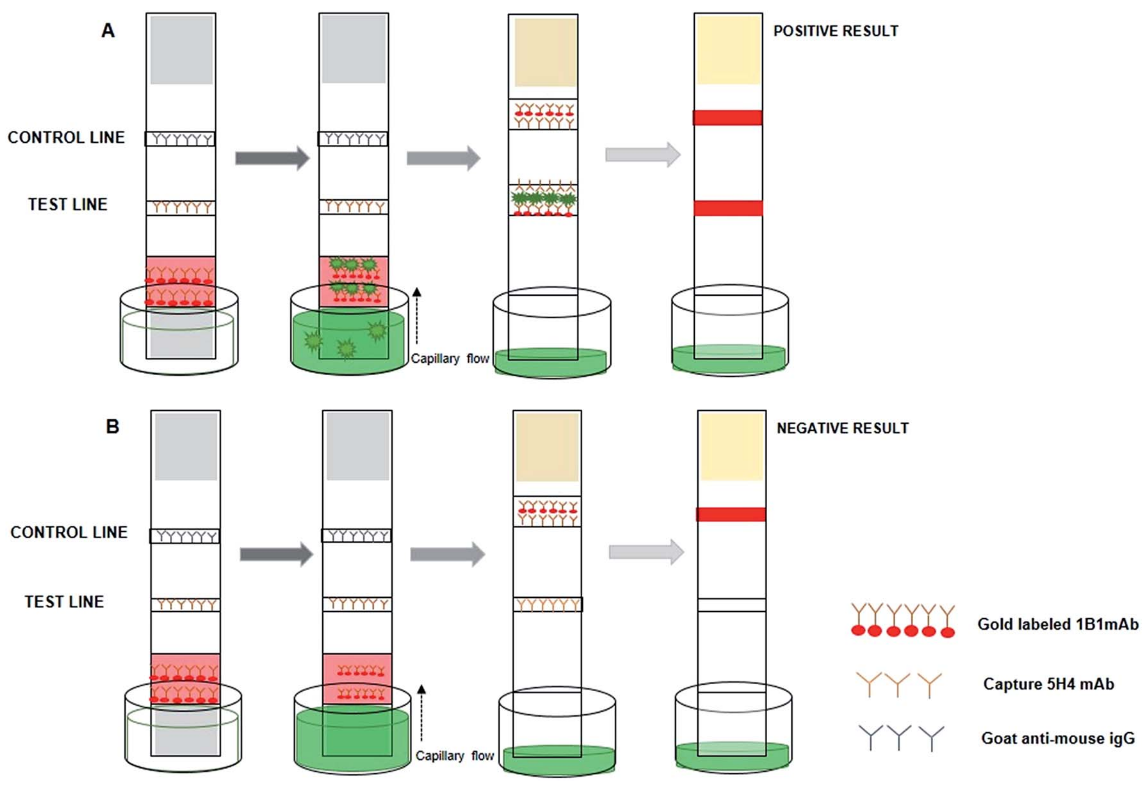

Fig. 5 The schematic image of the assembled strip test and the principle of detection using the sandwich immunoassay format (A) samples with Cry1Ac or Cry8Ka5 protein. (B) Samples without Cry1Ac or Cry8Ka5.

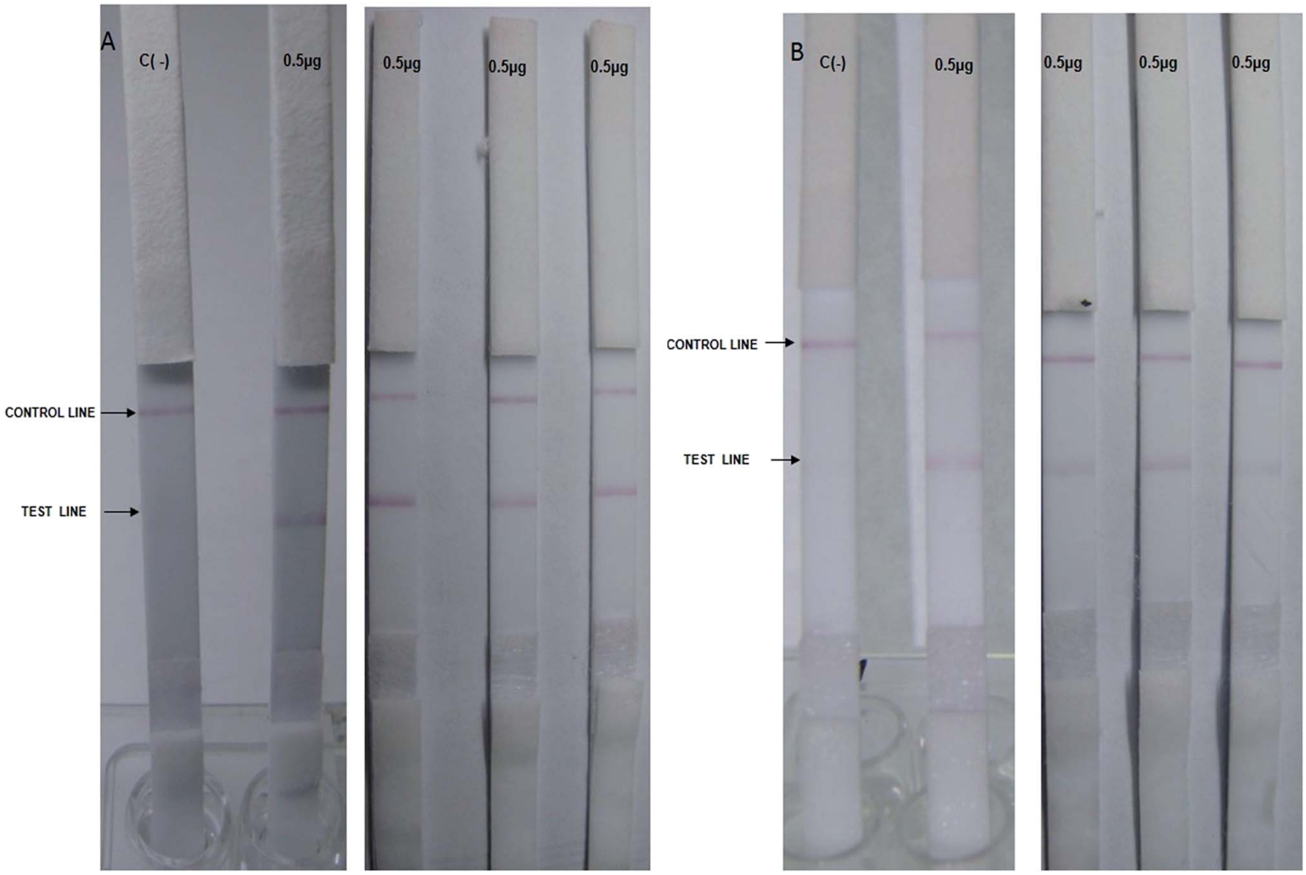

Fig. 6 Immunochromatographic strip test for Cry1Ac and Cry8Ka5 protein detection. Tests were evaluated using 0.5 μg of Cry1Ac toxin (A) and 0.5 μg of Cry8Ka5 toxin (B). (−) Corresponds to the negative control of 0.05 M carbonate–bicarbonate without the Bt proteins, pH 9.6 buffer (200 μL).

Development and optimization of the strip test

In this study, we developed a test strip based on a sandwich immunoassay format for qualitative detection of Cry1Ac and Cry8Ka5 proteins found in transgenic plants (Fig. 5). The system in the sandwich format employs two different antibodies that bind to distinct epitopes of the analyte. A labeled antibody is placed onto a glass-fiber membrane (conjugate pad) to serve as the detector reagent and another antibody is sprayed at the test line of the nitrocellulose membrane to serve as the capture reagent. A second antibody specific to recognize the first antibody is used to produce a control signal.[21]

As shown in Fig. 6, the strip tests were placed into the wells of microplates carrying test samples and the test results interpreted as positive or negative based upon visual inspection. As the sample flowed sequentially through the detection antibody (1B1 mAb) and the capture antibody (5H4 mAb), the Cry1Ac protein (Fig. 6A) or Cry8Ka5 (Fig. 6B) accumulated on the test line, to reveal a visible red line. A second red line was also observed on the control line, indicating correct test performance. A solution containing only extraction buffer without trypsin was used as the negative control. With this solution, no color developed on the test line, indicating the absence of Cry1Ac or Cry8Ka5 proteins. These results indicated that the developed strip test enabled specific detection of Cry1Ac or Cry8Ka5 in GM samples, showing high sensitivity levels.

Validation of strip test using GM cotton samples

Bollgard® cotton (positive control) and Coker 312 (negative control) samples were previously identified by using a commercial strip test produced by Envirolologix Inc. (data not shown). Cry1Ac and Cry8Ka5 toxin concentration levels from GM cotton leaf samples were estimated using a previously established standard curve (Fig. 7A). The standard concentrations of Cry1Ac and Cry8Ka5 toxins (0 to 1 μg) resulted in a standard curve with a good linearity of $R^2 = 0.9714$ and 0.9862, respectively. The cotton plants developed by our team were analyzed by PCR assays (data not shown) and two positive plants (named plant 50 and 217) were further characterized by ELISA. As shown in Fig. 7B, only plant 50 showed a statically significant difference ($p < 0.05$) to the non-GM cotton plant. Therefore, this plant was chosen for use in determining the accuracy of the strip test.

According to previous study reports,[37] the level of expression of the Cry1Ac protein in terminal leaf tissues was estimated to be 2.98 ng mg^{-1} or 2.98 μg g^{-1} dry tissue. In this study, we used 100 mg (dry tissue) of total protein extract from GM and non-GM cotton leaves. After the extraction procedure, the

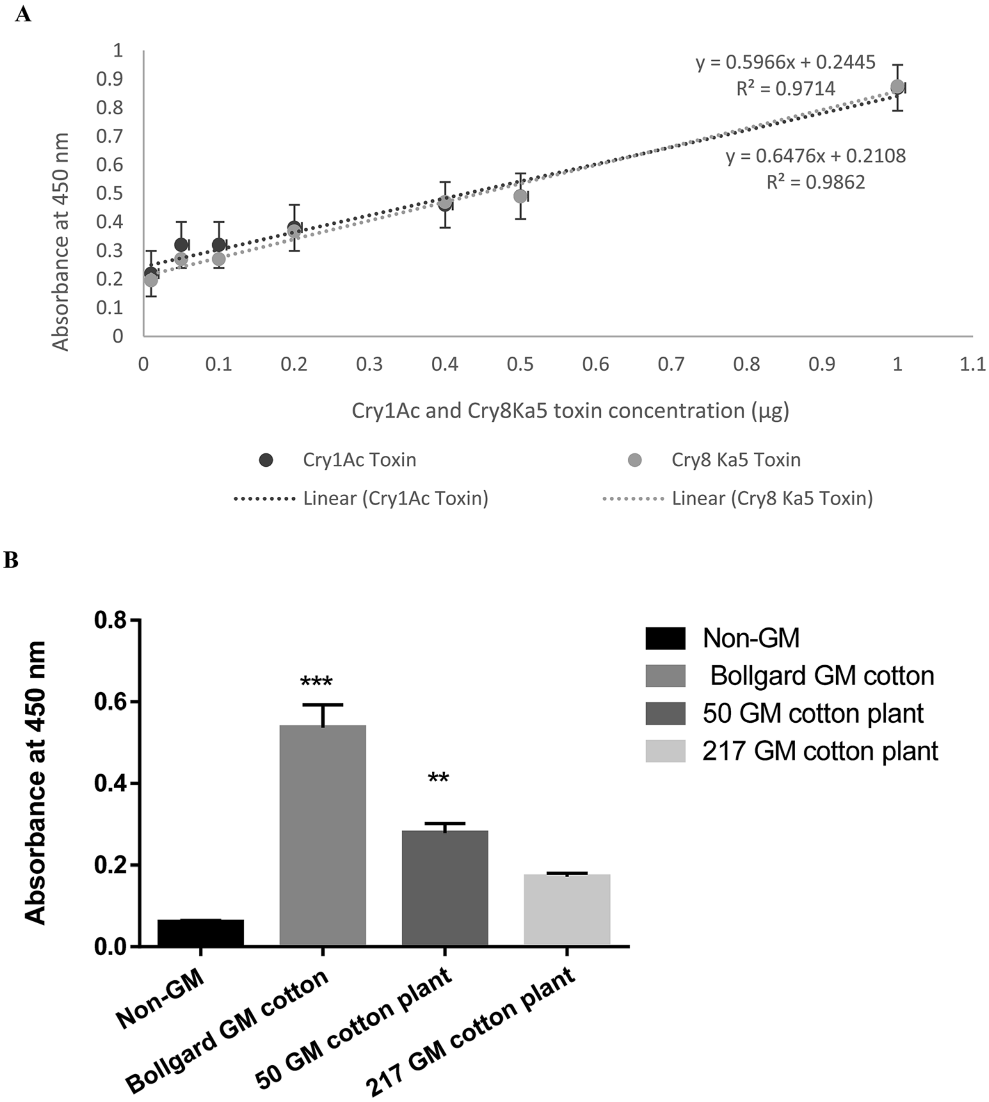

Fig. 7 Evaluation of accuracy of the strip test using GM cotton leaf samples. (A) Standard curve of Cry1Ac and Cry8Ka5 (0–1 µg) toxins using 1B1mAb. (B) Detection of Cry1Ac protein (Bollgard® GM cotton) used as a positive control and Cry8Ka5 protein (GM 50 and GM 217 cotton plant developed by our team). Non-GM (Coker 312) was used as a negative control. ***All statistical tests were performed using a two-way ANOVA. ***Denotes $p < 0.05$.

estimated amount of the Cry1Ac and Cry8Ka5 transgenic proteins in the sample was 0.298 µg mL^{-1}. For evaluation of the strip test, an aliquot of 200 µL of extract was used, giving an approximate concentration of 0.06 µg for Cry1Ac and Cry8Ka5.

As shown in Fig. 8, two red lines are clearly visualized in the test and control lines when all GM samples were applied to the strip test. This means that the strip test developed in this study reacts with Cry8Ka5 and Cry1Ac proteins found in these GM cotton samples (Fig. 8A and B, respectively). As expected, only a strong red line on the control line was observed when the non-GM samples (Coker 312) were applied to the strip test.

Currently, strip tests are commercially available to detect Bt transgenic proteins in GM cotton events used commercially to control lepidopterans.[22] These events include Bollgard® (expressing the Cry1Ac protein), Bollgard II® (expressing the Cry1Ac and Cry2Ab proteins) and Widestrike® (expressing Cry1Ac and Cry1F proteins). Now, however, no strip tests are available for the detection of the Bt Cry8Ka5 protein, which is used for the development of Brazilian GM cotton effective against the cotton boll weevil coleopteran insect pest, which is particularly harmful in Latin America.[8]

Conclusion

In the present study, we developed a qualitative immunochromatographic strip test for the detection of Cry1Ac and Cry8Ka5 proteins found in insect-resistant GM crops. The results showed that the strip test was sufficiently sensitive and accurate for the detection of these proteins in GM cotton crops. Moreover, these results were obtained within 10 min without the need for expensive equipment or reagents. The strip test is applicable for use directly in the field, as a rapid and cost-effective screening tool for Cry1Ac or Cry8Ka5 protein detection in GM crops.

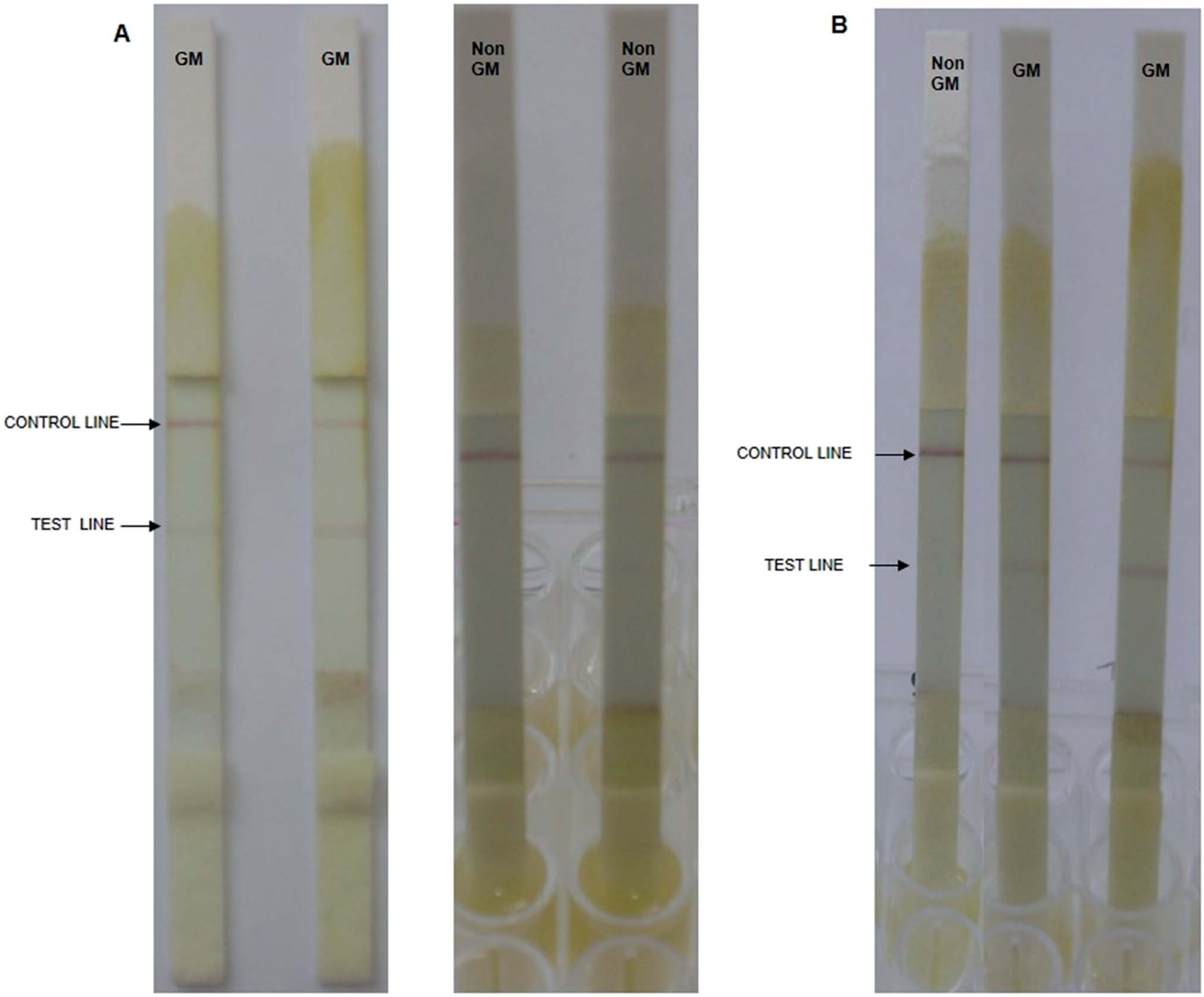

Fig. 8 Evaluation of the strip test for the detection of Cry8Ka5 and Cry1Ac toxins in GM and non-GM cotton leaf samples. (A) GM cotton leaf samples (developed by our team) containing the Cry8Ka5 toxin (B) Bollgard® cotton leaf samples containing the Cry1Ac protein. Non-GM cotton leaf samples (Coker 312).

Conflict of interest

All authors declare that they have no conflict of interest.

Acknowledgements

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